

CLAIM AMENDMENTS

1. (currently amended): A method ~~of generating~~ to generate both a cell-based and humoral immune response to a target polypeptide in an animal ~~in which an aqueous liposomal composition is administered subcutaneously or intramuscularly to the animal, the, which method comprises administering subcutaneously or intramuscularly to the animal~~ a composition comprising liposomes suspended in an aqueous liquid and a polynucleotide comprising a promoter operatively linked to a nucleotide sequence encoding said target polypeptide, having

wherein the liposomes have diameters in the range 100 to 2000 nm and comprising comprise a lipid bilayer and an aqueous intravesicular space, the lipid bilayer being formed of liposome forming components and

wherein said polynucleotide is entrapped in the aqueous intravesicular space comprising a polynucleotide in the form of a plasmid including a promoter and operatively encoding said target polypeptide wherein the target polypeptide is an antigen or a fragment of an antigen of an infectious microbe, whereby the said polynucleotide is delivered to and is expressed in target cells, to form target polypeptide and an immune response including an IgG response and Th1 and Th2 responses to the target polypeptide follows,

wherein said lipid bilayer includes at least one cationically charged component in an amount such that the lipid bilayer has an overall cationic charge;

whereby said polynucleotide is delivered to and is expressed in target cells whereby an immune response including an IgG response and Th1 and Th2 responses to the target polypeptide result;

wherein said polynucleotide is administered in an amount sufficient to elicit said immune response.

2-5. (canceled)

6. (currently amended): [[A]] ~~The method according to claim 1 comprising the preliminary step of providing said aqueous liposomal, wherein said composition has been prepared~~

by a process ~~in which an aqueous suspension of empty liposomes formed from the liposome forming components is provided, the~~ ~~that comprises~~

~~mixing~~ an aqueous suspension of empty liposomes ~~is mixed~~ with said polynucleotide to form a mixed suspension,

~~dehydrating~~ the mixed suspension ~~is dehydrated~~ to form a dehydrated mixture, and

~~rehydrating~~ the dehydrated mixture ~~is rehydrated~~ in an aqueous ~~composition~~ ~~liquid~~ to form liposomes which are ~~dehydration - rehydration vesicles (DRVs)~~ containing the polynucleotide in the intravesicular space.

7. (currently amended): [[A]] ~~The method according to claim 6 in which the dehydration rehydration vesicles are subjected to a micro fluidization step, wherein said process further includes subjecting said polynucleotide-containing DRVs to microfluidization or extrusion.~~

8. (currently amended): [[A]] ~~The method according to claim 6 in which the dehydration is carried out by lyophilisation, wherein in said process said dehydrating is by lyophilizing.~~

9-10. (canceled)

11. (currently amended): [[A]] ~~The method of claim 1 according to claim 38 in which the, wherein the~~ cationic component is selected from the group consisting of DOTAP, BisHOP, DC-Chol and stearylamine.

12. (currently amended): ~~A composition according to The method of claim 1 in which the liposome forming components include, wherein the lipid bilayer includes~~ a phosphatidyl ethanolamine.

13. (currently amended): [[A]] ~~The method according to claim 1 in which the, wherein the~~ mean diameter of the liposomes ~~in said aqueous liposomal composition~~ is in the range of 200 to 500 nm.

14. (currently amended): [[A]] The method according to claim 1 in which, wherein said aqueous liposomal composition comprises 0.1 to 10 µg of polynucleotide per mg liposome forming components lipid bilayer.

15. (canceled)

16. (currently amended): [[A]] The method according to claim 1 in which, wherein the composition is administered intramuscularly.

17. (currently amended): A process for forming an aqueous suspension of liposomes having diameters in the range 100 to 2000 nm comprising the steps:

- a) providing an aqueous suspension of small unilamellar vesicles formed from liposome forming liposome-forming agents selected from the group consisting of lipids, cholesterol and non-ionic and cationic surface active agents, wherein at least one cationically charged component selected from cationic lipids and cationic surface active agents is present in an amount whereby the small unilamellar vesicles have an overall cationic charge;
- b) adding to the aqueous suspension of small unilamellar vesicles a polynucleotide in the form of plasmid DNA nucleic acid including a promoter [[and]] operatively linked to a nucleotide sequence encoding an immunogenic polypeptide which is an antigen or a fragment of an antigen of an infectious microbe useful to induce an immune response in an animal to form a mixed suspension in which the weight ratio of liposome forming components making up the small unilamellar vesicles in step (a) to the polynucleotide nucleic acid added in step (b) is in the range (50 to 10000):1;
- c) dehydrating the mixed suspension to form a dehydrated mixture; [[and]]
- d) rehydrating the dehydrated mixture to form an aqueous suspension of liposomes that are dehydration-rehydration vesicles (DRVs) containing said nucleic acid in an intravesicular space thereof; and
- e) optionally subjecting the aqueous suspension of dehydration-rehydration vesicles to a further step of DRVs to microfluidisation whereby said aqueous suspension of liposomes is produced.

18. (currently amended): [[A]] The process according to of claim 17 further comprising the further step of subjecting the suspension of dehydration rehydration vesicles to a separate step in which removing non-entrapped polynucleotide is separated nucleic acid from the aqueous suspension of dehydration rehydration vesicles DRVs.

19. (currently amended): [[A]] The process according to of claim 18 in which wherein the level of non-entrapped polynucleotide nucleic acid separated from the suspension is in the range 10 to 90% based on polynucleotide added in step (b).

20. (currently amended): [[A]] The process according to of claim 19 wherein [[the]] said level is in the range 15 to 80%.

21-27. (canceled)

28. (currently amended): [[A]] The process according to of claim 17 in which, wherein the cationic component is selected from the group consisting of DOTAP, BisHOP, DC-Chol and stearylamine.

29. (currently amended): [[A]] The process according to of claim 17 in which, wherein the liposome forming components include a phosphatidyl ethanolamine.

30. (currently amended): [[A]] The process according to of claim 17 in which, wherein the small unilamellar vesicles in step (a) have a diameter in the range 100 to 400 nm.

31. (currently amended): [[A]] The process according to of claim 17 in which, wherein the dehydration-rehydration vesicles produced in step d) have diameters in the range 200 to 2000 nm.

32-33. (canceled)

34. (currently amended): A composition for administration to an animal to induce a cell-based and humoral immune response comprising to a polypeptide, which composition comprises liposomes having diameters in the range 100 to 2000 nm and having lipid-bilayers surrounding aqueous intravesicular spaces and a polynucleotide comprising a promoter operatively linked to a nucleotide sequence encoding said target polypeptide,

which [[the]] lipid-bilayers are formed from liposome forming components selected from the group consisting of glycerides, cholesterol and non ionic and cationic surface active agents including that include at least one cationic component selected from cationic surface active agents and cationic lipids in an amount to confer an overall cationic charge on the liposome forming components, and

wherein the polynucleotide is entrapped in the aqueous intravesicular space is aqueous and contains a polynucleotide in the form of a plasmid having a promoter and operatively encoding an antigen or a fragment of an antigen of an infectious microbe, wherein the target microbe is a virus.

35. (currently amended): [[A]] The composition according to claim 34 in which the virus is selected from of claim 39, wherein the viral polypeptide is a polypeptide of hepatitis B, hepatitis C, influenza [[and]] or human immunodeficiency virus.

36. (currently amended): [[A]] The composition according to claim 34 in which the polynucleotide encodes of claim 35, wherein the viral polypeptide is hepatitis B surface antigen or haemagglutinin.

37. (currently amended): [[A]] The composition according to claim 34 which is an aqueous composition in which of claim 34, wherein the liposomes are suspended in a pharmaceutically acceptable aqueous vehicle.

38. (canceled)

39. (new): The composition of claim 34, wherein the polypeptide is a viral polypeptide.